Food and Agriculture Organization of the United Nations

Surveillance of Banana *Fusarium* Wilt TR4

(Detection Survey Protocol for FOC-TR4)

Endorsed by NPPO-Nepal on 13 Dec., 2023

Contents

1. Background information

1.1 Purpose and scope of the survey

Fusarium oxysporum f. sp. *cubense* (FOC TR4), a fungus that causes notorious disease tropical race 4 (TR4), was recently reported in Nepal in the Tikapur area of Kailali district and Kathahawa of Pratapur Rural Municipality, Nawalparasi West in 2023. The environment in Nepal's banana-growing areas is conducive to the disease's survival and spread. In its initial quick survey of the Tikapur and Pratappur banana fields, NPPO-Nepal clearly confirmed that the disease is spreading rapidly throughout banana orchards. The *Fusarium oxysporum* f. sp. *cubense* (FOC) Tropical Race 4 (TR4) pathogen is the cause of banana wilt, which is an imminent threat to the banana production in Nepal. Considering the seriousness of the problem, implementing a thorough survey and surveillance technique is essential to precisely tracking and managing the spread of banana wilt TR4. The development and implementation of an effective protocol would facilitate early detection, prompt response, and the use of preventative measures—all of which are essential for ensuring the sustainability of the banana production in Nepal.

1.2 Target pathogen

Taxonomic tree of the pathogen is presented below (CABI, 2023).
Domain: Eukaryota
Kingdom: Fungi
Phylum: Ascomycota
Subphylum: Pezizomycotina
Class: Sordariomycetes
Subclass: Hypocreomycetidae
Order: Hypocreales
Family: Nectriaceae
Genus: *Fusarium*
Species: *Fusarium oxysporum* f.sp. *cubense* tropical race 4

2. Host range of FOC TR4

Banana (*Musa spp.*), and wild banana (*Musa acuminata*) (family Musaceae) are the major host of the pathogen. *Musa balbisiana* and *Musa textilis* (Manila hemp) (family Musaceae), *Heliconia caribaea, H. mariae, H. psittacorum* (family Heliconiaceae) are the other host plants of the pathogen (CABI, 2023). Nearly

all varieties of bananas, including the main commercial variety, Cavendish are susceptible to Panama TR4. Some weeds and grasses can also be infected with Panama TR4, though no disease symptoms are externally expressed.

TR4 is strongly pathogenic to the commercially important banana cultivars of the group Cavendish (AAA) such as Grand Naine, Williams and Valery, among others. Additionally, susceptible cultivars to race 1, race 2 and subtropical race 4 are often susceptible to TR4 (Ploetz et al., 2015; García-Bastidas, 2019). TR4 is also a risk for Musa species from different banana sections within the family Musaceae (Pérez-Vicente et al., 2014; Cheng et al., 2019).

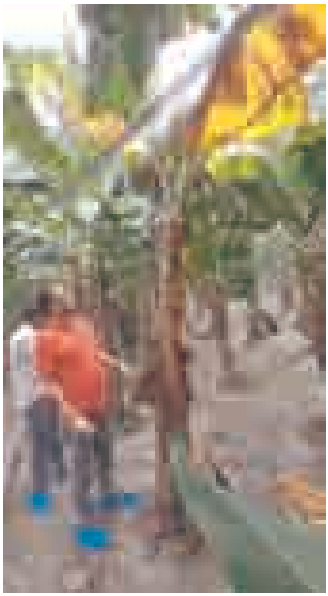
3. Mode of dispersion

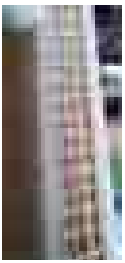
- A common means of dispersal is the planting of infected suckers in a healthy field. These suckers may not exhibit symptoms, and so might be easily replanted without it being known that they are infected.
- Infected soil attached to plant material, farm machinery and tools, vehicles or footwear is also a source of the fungus. If spores get in to irrigation water stores, such as reservoirs, they can also spread to new plantations when the contaminated water is used for irrigation.
- Additional risks have been associated with domestic animals (pigs, sheep, etc.) which have the potential to transport inoculum from an infected plantation to a plantation of healthy plants.
- **Natural Dispersal:** TR4 produces micro and macroconidia, as well as chlamydospores which can spread via surface water, rivers and streams. The spread of the pathogen by water can be significant, for example, with irrigation/flooding.
- **Vector Transmission (biotic):** TR4 has been detected on the exoskeletons of the banana weevil, *Cosmopolites sordidus* (Ploetz, 2015; Pegg et al., 2019). Annex #
- TR4 spreads mainly by passive means, this occurs by involuntary movement of the pathogen by biotic or abiotic means. Active movement can be limited by quarantine measures. However, once the pathogen is in the soil, it is virtually impossible to eradicate.

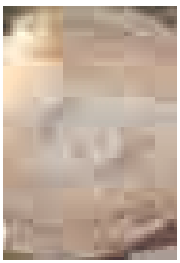
4. Disease symptoms

- Yellowing of leaf margins and at the base of older leaves, Entire leaves turning yellow and the leaf margins turning brown.

FOC







5. Timing of survey

The survey will be carried out round the year. The priority will be given during the hot and humid months (May- July).

6. Selection of survey area

As per the requirement of NPPO Nepal.
(To begin with commercial banana growing districts)

Table 1. Fifteen top most banana growing districts in Nepal

S N	Districts	Area under banana 9 (ha.)	Production (mt.)	Productivity (mt./ha.)
1	Nawalparasi West	2900	30866	14.03
2	Chitwan	2534	47187	21.40
3	Morang	2481	27956	14.91
4	Sarlahi	2015	25693	16.20
5	Jhapa	1890	34595	18.50
6	Saptari	1780	25934	15.30
7	Rautahat	1550	9300	15.50
8	Kailali	910	16667	20.45
9	Bardiya	800	12950	18.50
10	Gorkha	753	10240	16.46
11	Kanchanpur	550	8925	17.50
12	Bara	545	5063	15.00
13	Sunsari	514	8926	18.87
14	Dhanusa	350	5413	15.60
15	Rupandehi	280	5350	20.42

Source: MoALD, 2023

7. Number of plants for observation

All the banana orchards in the suspected area should be monitored.
Each fourth row of banana plantation in each orchard should be observed.

8. Plant parts to be observed

- Leaves
- Pseudostem (external and internal)
  - Vascular part (precaution should be provided to prevent spread)
- Whole plant

9. Sample collection and preparation from the disease suspected host plant

The sample should consist of a section from the pseudostem of the wilted banana plant where typical continuous discoloured vascular strands are evident. The sample should be taken from as low in the pseudostem as is possible but not from areas where decay is advanced. Also, the sample should be taken from as close to the centre of the pseudostem as is possible, as opposed to the outermost leaf bases.

As banana tissue is very wet, the risk of bacterial contamination of samples is high, particularly in warm weather and samples can deteriorate rapidly. The chance of recovering healthy cultures of *Fusarium oxysporum* f. sp. *cubense* (FOC) decreases as the sample deteriorates.

Samples should be kept in heavy paper bags or wrapped in paper until the strands can be excised. Avoid plastic bags as this causes the samples to sweat and promotes growth of bacteria.

10. Diagnostic laboratory

- National Plant Pathology Research Centre, Khumaltar, Lalitpur
- Central Agricultural Laboratory, Hariharbhawan, Lalitpur
- Potential laboratory
- Agriculture and Forestry University, Chitwan
- TU/IAAS
- Plant Protection Laboratory, Pokhara

** Biosecurity protocol to handle the quarantine sample should be followed in each laboratory.*

11. FOC TR4 diagnosis protocol

11.1 Isolations of FOC in Laboratory

Isolation of the fungus from plant diseased collected material

- FOC is generally isolated from colored strands which are developed due to vascular discoloration of banana pseudostem. It is always advised to take samples from the upper part of the plants as much as possible.
- The isolation can be attempted as soon as the strands with vessels are dry (possibly the day after the collection)
- Cut the infected part into small pieces (3-6mm long)

- Surface sterilization with 70% ethanol or 0.5% sodium hypochlorite for 30 second followed by washing with sterile distilled water twice under laminar flow hood.
- Drying the samples either blotting with sterile tissue paper or over tissue paper
- Plate the tissue section with vascular vessels in Petri plates with either acidified potato dextrose agar (39 gm PDA in 1000 ml water, add 750 µl lactic acid) or ¼ strength PDA or water agar (WA)
- If Fusarium is present, it will grow out from the vessels in 2-4 days.
- Prepare single conidia cultures of each specimen.

11.1.1. Single spore isolations (single conidia)

- *Fusarium oxysporum* single spore isolations are obtained by the plate dilution method and streaking plates (showed ahead).
- Collect a scrape of sporulating hyphae from cultures growing on acidified PDA and dissolve in 10 mL sterile distilled water in test tubes.
  - From an initial suspension, a dilution serial can be prepared.
- Pipette or streak 1 mL of each of the dilutions on water agar.
  - Incubate plates overnight at 25°C with caps in upside position
  - Check the plates under a dissecting microscope the following day to localize germinated conidia and transfer with a sterile needle or scalpel single conidia isolated from the water agar to new 90 mm plates with ¼ strength PDA.
  - Additionally, single-spore cultures can also be obtained by dissecting the tip of a single growing hypha of an old culture grown in carnation leaf agar (CLA).

PDA supplemented with streptomycin. Proceed to melt the required number of 240 mL PDA bottles in a water bath. When media has melted, place the bottles in a water bath at 50°C for 20 min or until the media reaches 50°C. For each 240 mL of media, add 1.2 mL of streptomycin solution (1g of streptomycin sulfate powder to 100 mL distilled water) just before dispensing the media in the Petri plates.

11.1.2. Single spore isolation and preservation

- Re-culture isolates on PDA plates for single spore isolation.
- Do serial dilution of spores and plate dilution factors 10^{-2}, 10^{-3} and 10^{-4} on 1.5 % water agar.

- Isolate germinating spores by marked the bottom of the agar plates after observing under 10× magnification of a compound microscope.
- Transfer the marked agar block into fresh PDA plates using sterile fine tip needle
- After growth on PDA, a block of approximately 2×2 mm from each mono-conidial culture is transferred onto PDA slants for medium-term preservation.
- For long-term preservation, three sterilized cellulose paper strips measuring 3×1 cm^2 should be placed in each slant. After fungal colonizes the paper, the strips will be dried in the room temperature for 5-7 days and should be preserved in a sterilized envelop at -20 ºC (Stocco et al., 2010).

11.2 Morphological features of FOC

FOC cannot be morphologically distinguished from other formae speciales that cause wilting in other hosts and other non-pathogenic *F. oxysporum* endophytes, saprophytes and antagonists. FOC is an anamorphic fungus without a known sexual stage (teleomorph). The fungus produces macroconidia, microconidia and chlamydospores for reproduction and dispersal. Macroconidia and microconidia are produced in orange structures called sporodochia. Macroconidia (27-55 × 3.3-5.5 µm) are abundant, falcate to erect to almost straight, of thin walls, with 3 to 5 septa (usually 3 septa). Apical cell is attenuated or hook shaped in some isolates. Basal cells are foot shaped. Macroconidia are developed in single phialids in hypha (Figure 3A). Microconidia (5-16 × 2.4-3.5 µm), usually without septa, can be oval, elliptic to kidney shaped and developed abundantly in false heads in short monophialides (Figures 1 A, 1B and 1C). Chlamydospores (7-11 µm diameter), are abundantly formed in hyphae or in conidia, single or in chains, usually in pairs, but their development can be slower in some isolates **(Figure 3D).** On potato-dextrose-agar (PDA) medium, colonies have a variable morphology. Mycelia can be hairy to cottony, spaced or abundant and variable from white, salmon, to pale violet. Black to violet sclerotia can be produced in some isolates. *Fusarium oxysporum* usually produces pale violet to dark red color pigments in PDA (Stover, 1962; Ploetz, 1990; Pérez-Vicente et al., 2003). Some isolates mutate rapidly from pionnotal (with abundant greasy or brilliant conidia aggregates) to a flat humid mycelia of white-pale yellowish to peach color on a PDA culture (Stover, 1962; Ploetz, 1990). In modified Komada media (K2), some isolates of TR4 develop laciniated radial colonies, which are not found in isolates of races 1 and 2 (Sun et al., 1978; Qi et al., 2008). However, this characteristic is not a determinant of a FOC TR4 diagnostic.

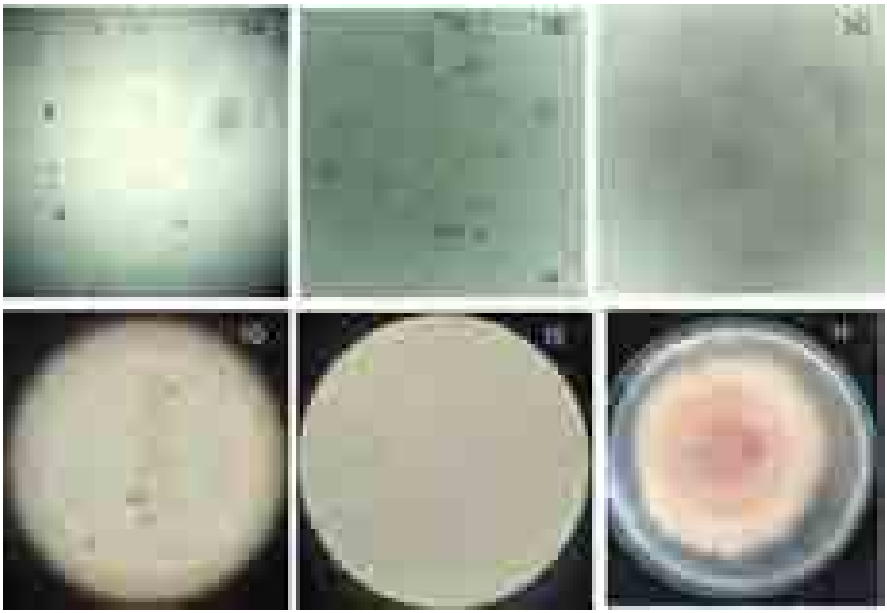


Figure 1: Reproductive structures of *Fusarium oxysporum* f. sp. *Cubense* A. Microconidia B. Macroconidia, C. Microconidia D. Chlamydospores. E. Micro-, macro-conicida and chlamydospore in one place. F. *Fusarium oxysporum* f. sp. *cubense* tropical race 4 in PDA media

11.3 Pathogenicity test

TR4 could be detected by pathogenicity test. However, the pathogenicity test should be always done in controlled environment with high biosafety and security under BSL2 or higher-level greenhouse conditions to avoid the environmental release.

Use acclimatized 45-days-old tissue-culture plantlets of a susceptible variety Malbhog' (AAB), Willam hybrid and G9. Check the reaction to FOC of a set of banana genotypes on Table 1.

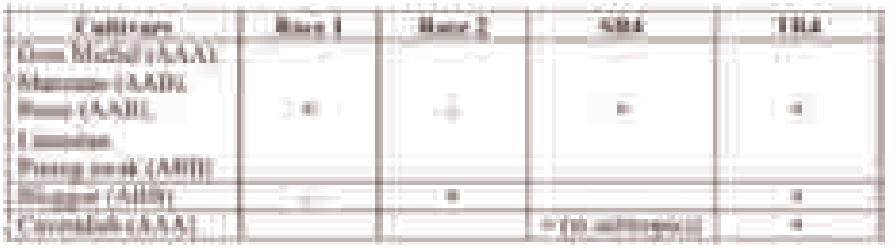
Observations: Plants should be grown in a disease-free environment with no contact with FOC, which may eventually provoke accidental or cross-contamination. Plants should be at least 15-25 cm high and shows no nutrient deficiency.

11.4 Inoculum production

- Inoculate the FOC single-spore isolates to be studied on Petri plates with Potato Dextrose-Agar (PDA) for one week .

6

***Fusarium oxysporum* f. sp. cubense**



FOC

FOC

FOC

A.

sequencing. The protocol utilizes organic solvents and the harmful nature of some of the solvents, combined with the relatively long time to complete the protocol, can therefore be a limitation of using this method.

- Using a sterile scalpel, scrape ~50 mg of mycelium from agar.
- Homogenize tissue
  - Add 200 µL CTAB buffer to sample and grind tissue with a micropestle (1.5 mL Eppendorf tubes often work best)
  - Add 200 µL CTAB buffer to sample and grind tissue with a pipette tip with the end melted
  - Place end of a 1000 µL pipette tip into the flame of an ethanol or Bunsen burner until the hole is sealed
  - Allow the tip to cool. The plastic end often becomes cloudy as it cools

Homogenize using a Qiagen Tissue Lyser (or other lysis machine)

- Add glass beads or a heavy metal bead to the tube (2 mL Eppendorf tubes often work best for the heavy beads)
- Run Tissue Lyser at 24 Hz for four minutes
- Add 500 µL CTAB buffer, 7 µL Proteinase K, 7 µL RNase A and 7 µL β-Mercaptoethanol.
  - Incubate on a rotor at 65°C for at least 3 h (1 h if samples are freeze-dried).
  - Centrifuge for 5 min at 20,000×*g.*
  - Transfer supernatant to a new tube.
  - Add 700 µL phenol-chloroform-isoamyl alcohol (25:24:1).
  - Mix by turning upside down 100 times and incubate for 5 min at 18°C–27°C.
    a. Vortexing for 5 s is also possible but increases the risk of shearing DNA
  - Centrifuge for 30 min at 20,000×*g* and 20°C.
  - Transfer the upper phase, avoiding the white interphase, to a new tube.
  - Add 700 µL chloroform:isoamyl alcohol (24:1).
  - Mix by turning upside down 100 times and incubate for 5 min at 18°C–27°C (Vortexing for 5 s is also possible but increases the risk of shearing DNA)
  - Centrifuge for 15 min at 20,000×*g* and 20°C.
  - Transfer upper phase, avoiding the white interphase, to a new tube while measuring supernatant volume.

- Add 1/3 supernatant volume of 5M NaCl and 2/3 supernatant volume of ice-cold isopropanol to the tube. (This step brings the DNA out of solution. While the isopropanol does not have to be ice-cold, it helps to improve overall yield).
- Mix by turning upside down 20 times and centrifuge for 30 min at 20,000×*g* and 20°C.
- Discard supernatants (Avoid disturbing any pellet with a pipette tip).
- Add 750 µL 70% Ethanol and centrifuge for 3 min at 20,000×*g.*
  a. The use of ice-cold ethanol can improve yield.
  b. Can be repeated to increase purity.
- Discard supernatants.
  a. Avoid disturbing any pellet with a pipette tip.
- Air-dry pellets then add 50–100 µL EB buffer (or other elution buffer).
  a. Maximum time for air-drying is 10 min. Be sure to properly remove ethanol with a 10 µL pipette and the remaining ethanol will evaporate within a few minutes.
  b. Allow the sample to dissolve for at least 1 h at 18°C–27°C or for 12 h at 4°C.

Note: Unless otherwise indicated, centrifugation steps are at 18°C–27°C.

C. Wizard® Genomic DNA Purification Kit (Promega kit)
Isolation of Genomic DNA from Fungal Cultures
 # Prepare Fugal Lysate

- Grind approximately 40mg of fungal mycelia tissue in liquid nitrogen.
- Add 600µl of Nuclei Lysis Solution. Incubate at 65°C for 15 minutes.
- Add 3µl of RNase Solution. Incubate at 37°C for 15 minutes. Cool sample to room temperature for 5 minutes. Proceed to Protein Precipitation and DNA Rehydration, Step 1 (below).

 # Protein Precipitation and DNA Rehydration

- Add 200µ Protein Precipitation Solution. Vortex.
- For yeast only: Incubate 5 minutes on ice
- Centrifuge at 13,000–16,000 × g* for 3 minutes,
- Transfer supernatant to clean tube containing room temperature 600µl isopropanol
- Mix by inversion and centrifuge at 13,000–16,000 × g* for 2 minutes

- Decant supernatant and add 600µl room temperature 70% ethanol.
- Centrifuge at 13,000–16,000 × g* for 1 minutes.
- Aspirate the ethanol and air-dry the pellet.
- Add 100µl DNA Rehydration Solution.
- Rehydrate at 65°C for 1 hour or overnight at 4°C.

E. SeraSil magnetic beads technique

1. **Scrape mycelium from agar plate, grind in liquid nitrogen.**
2. Add 400µl lysis buffer to the sample tube and wait for 20 minutes (how to make lysis buffer is explained on ANNEX 6). Invert the tubes 2-3 times during this time.
3. Centrifuge in a benchtop micro-centrifuge for 05m00s (at ~6000 rcf) to gather the cell debris at the bottom of the microfuge tube (or let the contents settle for 2m00s).
4. Transfer 300µl of the supernatant to a new 1.5ml microfuge tube, leaving the cell debris behind.
5. Prepare the SeraSil magnetic beads by shaking or vortexing them so they are not set at the bottom of the tube. Add 30µl of the SeraSil beads and 360µl of the Binding buffer PB to the sample and mix by pipetting.
6. Transfer the microfuge tube to a magnetic rack and open the lid. Wait for roughly 02m00s for the beads to pellet and the supernatant to clear. !Do not remove the microfuge tubes from the magnetic rack until stated!
7. Remove and discard as much supernatant as you can without disturbing the pellet. This can be difficult, so carefully slide the pipette tip down the side of the tube (away from the beads) and draw up the liquid very slowly. Do not remove any beads. If beads are accidentally pipetted, return supernatant to the microfuge tube and allow to re-pellet before trying again.
8. Add 500µl of 80% ethanol without disturbing the pellet and leave to stand for 0m30s, then remove and discard the ethanol very carefully, without disturbing the pellet of beads.
9. Repeat step 5.
10. Leave the tube containing the beads on the rack to dry for 05m00s, or until no drops of ethanol are left.
11. Remove the microfuge tube from the magnetic rack and add 50µl of elution buffer EB. Gently re-suspend beads in solution by pipetting the buffer up and down, over the pellet. You are releasing the DNA from the magnetic beads, so it is important to wash the buffer over the beads multiple times to collect as much of the DNA in the solution as possible.

12. Incubate at room temperature with the lid closed for 5m00s.
13. Transfer the microfuge tube back to the magnetic rack and open the lid. Wait for the beads to pellet this should take roughly 2m00s.
14. Transfer supernatant to a new 1.5ml microfuge tube but avoid disturbing the beads. If beads are accidentally pipetted, return the supernatant and allow to re-pellet before trying again. This tube now contains DNA from your sample

 # Quantification of DNA

DNA yield (ng/µL) is quantified using a Qubit 4 Fluorometer with a Broad Range dsDNA assay kit (Thermo-Fisher, USA). Protein and RNA contamination is assessed using a Nanodrop 1000 (Thermo-Fisher, USA) spectrophotometer by comparing the absorption ratio for λ260: λ280 (260/280) while reagent contamination is also assessed using the Nanodrop by comparing the absorption ratio for λ260: λ230 (260/230). DNA integrity and fragment size is assessed qualitatively using agarose gel electrophoresis.

 # Polymerase chain reaction

Based on two single nucleotide polymorphisms present in the IGS region of FOC, Dita et al. (2010), developed a PCR-based diagnostic tool to specifically detect isolates from TR4 (VCG 01213). Primers FOC TR4-F (5´-CACGTTTAAGGTGCCATGAGAG-3´) and FOC TR4-R (5'-GCCAGGACTGCCTCGTGA-3') yield an amplification product of 462 bp specific to VCG 01213 (FOC TR4). Validation of the specificity of these primers involved TR4 isolates from different geographic regions, as well as FOC isolates from 19 other VCGs, other fungal plant pathogens and DNA samples from infected tissues of the Cavendish banana cultivar Grand Nain (AAA). Pérez et al. (2012), using primers developed from IGS sequences, developed a real time PCR diagnostic method able to differentiate isolates of FOC TR4, FOC R1 and FOC R2 races present in Cuba.

FOC

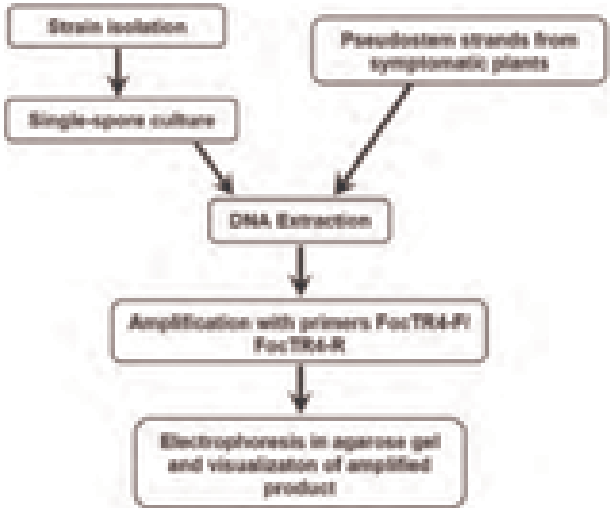


Figure 2 FOC

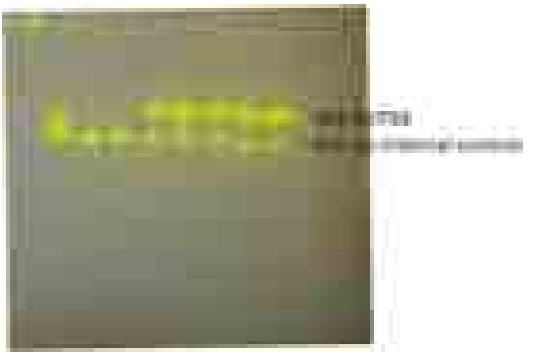
FOC

Reagent	Volume (
FOC	
FOC	
)	1

	Temperature	Time
1		
2		
3		
4	72	
	72	
6	12	

FOC

Amplicon size



FOC

Fusarium oxysporum

FOC FOC

FOC FOC FOC

Components	Final volume	
	15 µL	20 µL
DNA	3 µL	5 µL
primer combination*	3.0 µL (10 µM)	0.75 µL (10 µM)
SYBR	10 µL	7.5 µL
Water	6.0 µL	3.75 µL

*primer combination for TR4= qFocR4T-1 / qFocR4T-11

primer combination for R1/R2= qFocR1R2-f / qFocR1R2-r

DNA is amplified with primers qFOCR4T-f and qFOCR4T-r1 and the following program:

1) 95°C for 15 min.

2) 94°C for 15 sec.

3) 69°C for 30 sec. 30 cycles 30 cycles

4) 72°C for 30 sec.

In case of R1/R2 detection with qFOCR1R2-f and qFOCR1R2-r primers the program will be:

1) 95°C for 15 min.

2) 94°C for 15 sec.

3) 57°C for 30 sec. 22 cycles 22 cycles

4) 72°C for 30 sec.

Annex 1. Trace back and trace forward survey format for risk mapping and profiling for delimitation decisions

Area Tracking on the basis of following information is used in setting up of any official quarantine zone against Banana FOC TR4 in Nepal (Kailali, Kanchanpur, Bardiya, Janakpur, Bara, Rupandehi, Nawalparasi, Chitwan, Sarlahi, *and other areas of banana plantation*)

Name of the **hot spot:**

Banana grower's name, address and contact no:

Area and status of banana plantation

Trace		Back	Forward
Movement of sources of contamination	Planting Material		
	Animal		
	Soil		
	Water		
	Tractor and machinery		
	Product conveyance		
	Workers movement		
	Relatives movement		
	Presence of collateral host/s species of the family Musaceae and Heliconeasceae		
	Presence of non-host weed species. *Paspalum fasciculatum*, *Panicum purpurascens* [*Brachiaria mutica*] *Ixophorus unisetus*) *Commelina diffusa*		
	Presence of Vectors *Cosmopolites sordidus*		

Date:

Annex 2. Vector Transmission, *Cosmopolites sordidus*

Vector Transmission (biotic): TR4 has been detected on the exoskeletons of the banana weevil (BW), *Cosmopolites sordidus* (Ploetz, 2015; Pegg et al., 2019), and as much as 10% of weevils found contaminated with viable spores of TR4 on their exoskeletons (Meldrum et al;, 2013). So, the proved BW as a vector of TR4 should be taken into account in the detection survey along with of banana wilt TR4 in the banana commodity.

The banana borer/weevil (*Cosmopolites sordidus*) is up to 12 mm long. It feeds on the corm of the plant, weakening it and making it more likely that it would break at ground level and collapse over in the wind. Additionally, the damage also allows fungi to enter the tunnels causing rotting of the corm, and reduces yield (Heck, Alves & Mizubuti, 2021; Guillen et al. 2021).

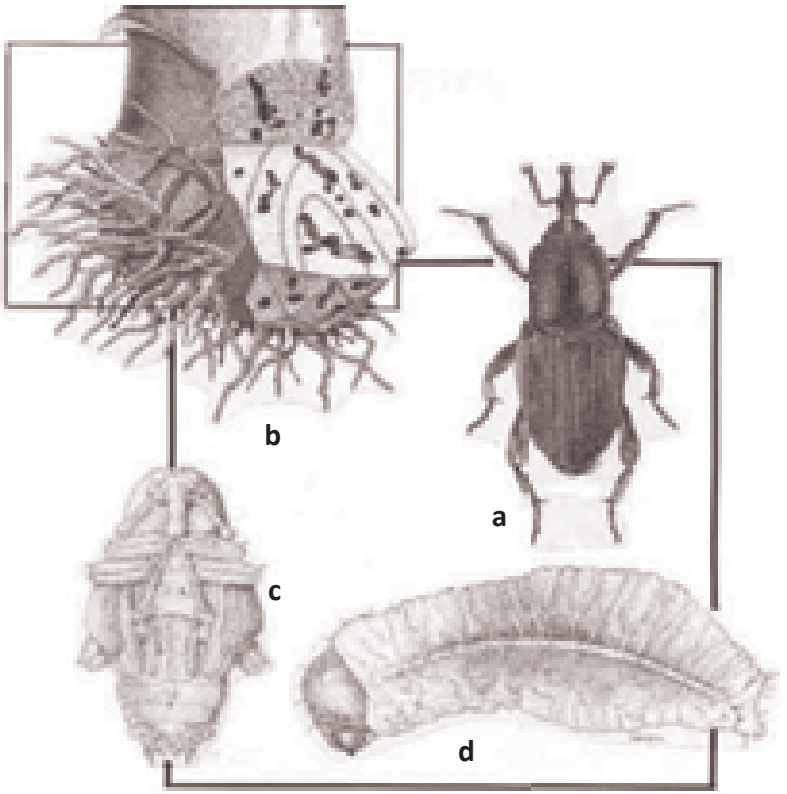
***Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae)**

Adult: The adult weevil is around 11 mm long, shiny, and dark brown to grey black in colour. Although it lacks the pronotum's depressions, it resembles billbugs (*Sphenophorus*) in overall appearance. The hook-like appendages on all tibiae allow the beetle to cling firmly to plant tissue.

Egg: Often deposited in the larger cell-like compartments in the tissue, eggs are laid around the corm, between the sheaths of the leaves, and between the stems. Typically, they are placed separately, with the recently hatched larvae penetrating the corm.

Larva: The head capsule is dark reddish brown, and the body is white, like the typical weevil subfamily Calendrinae. From a lateral perspective, the final two abdominal segments seem "chopped off" because of their modification into a plate-like structure. All other abdominal spiracles are small and hazy, with the exception of a big, elongate spiracle present in the eighth abdominal segment.

Pupa: The pupa is likewise characteristic of the Calendrinae subfamily, with several transverse depressions and a very irregularly margined beak.

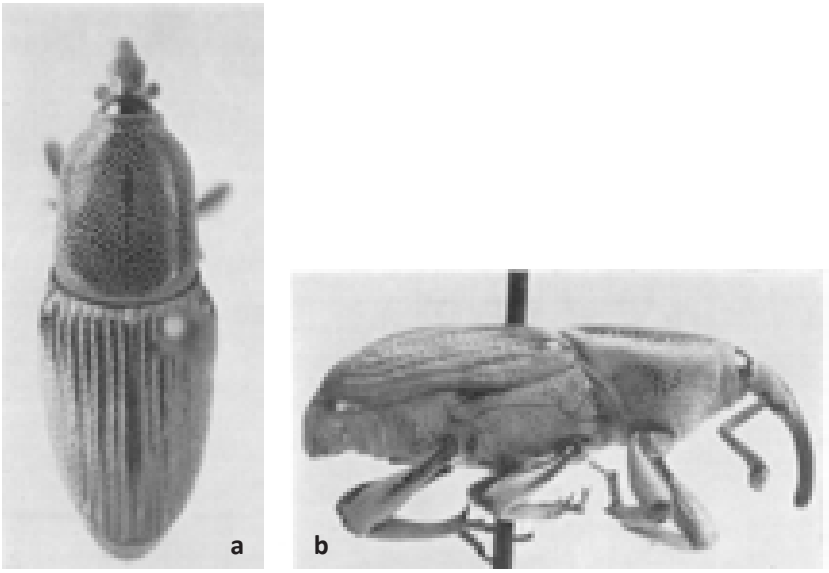

b
a
c
d

Trapping and monitoring of *C. sordidus*

Trapping of adults is often used to monitor weevil numbers, although interpretation of trap captures is difficult. The use of a "sandwich trap" using banana pseudostem as an attractant has been suggested. A maximum of ten weevils per trap per week have been captured using banana pseudostem disc traps, although the number of weevils collected at these traps was consistently too low to accurately assess weevil density (Gold & Tinzaara, 2008).

Taxonomic identity of *C. sordidus*

Highly polished disc of the pronotum. Elytra with striae mostly well impressed and some intervals appearing elevated; most intervals on dorsum in part distinctly raised and polished (polished areas bare of pruinosity), especially basad, and the elytra (in clean specimens) distinctly vittate



a. Dorsal view of *C. sordidus* **b.** Lateral view of *C. sordidus*

Source: (Zimmerman, 1968)

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